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FINAL REPORT

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OBJECTIVE: To identify the effects of moderate hydrostatic pressure on receptor and effector elements involved in transmembrane signal transduction, we examined the A_1 adenosine receptor - inhibitory G protein (G_i) - adenylyl cyclase signal transduction complex. Our experiments were designed to identify and define at the molecular level pressure effects on system components in isolation and on the entire functional complex.

APPROACH: Two marine teleost species, *Sebastolobus alascanus* and *S. altivelis* which live at different depths were used as a model to study pressure adaptation. This approach of parallel comparisons has permitted the identification of patterns of pressure adaptation. Among the techniques employed were assays of adenylyl cyclase activity, assays of the high-affinity GTPase activity of G proteins, antibody quantitation of G proteins, pertussis and cholera toxin ADP-ribosylation of G protein subunits, affinity labeling and peptide mapping.

ACCOMPLISHMENTS: We have characterized the effects of hydrostatic pressure on the A_1 adenosine receptor - inhibitory G protein (G_i) - adenylyl cyclase signaling complex. Hydrostatic pressure inhibits basal adenylyl cyclase activity, increases the K_m of ATP for adenylyl cyclase and decreases the efficacy of agonists in inhibiting cAMP accumulation. Pressure also affects the high-affinity GTPase activity in brain membrane preparations. There are two GTPase activities, one with a low- and one with a high-K_m of GTP. The high-affinity GTPase activity, characteristic of the α subunits of the guanine nucleotide binding regulatory protein (G protein) pool, was stimulated by the A_1 adenosine receptor and muscarinic cholinergic receptor agonists. High-affinity hydrolysis of GTP, measured at 0.3 μM GTP, was stimulated 22% in both species by 340 atm pressure. At 340 atm pressure, the apparent K_m of GTP is decreased approximately 10% in each of the species, and the V_{max} values are increased 11 and 15.9% in *S. alascanus* and *S. altivelis*, respectively. The apparent volume changes associated with the decreased K_m of GTP values and the increased V_{max} values ranged from -7.0 to -9.9 ml/mol. Increased pressure, however, markedly decreases the efficacy of adenosine and muscarinic agonists in stimulating GTPase activity. These results suggest that the effects of increased hydrostatic pressure on transmembrane signal transduction by this system may stem, at least in part, from pressure-increased GTP hydrolysis and the concomitant termination of inhibitory signal transduction.

Pertussis toxin-catalyzed [^{32}P]ADP-ribosylation at atmospheric pressure and 5°C was used to probe the guanine nucleotide binding regulatory proteins G_i and G_o in brain membranes from the two *Sebastolobus* species. The membranes from the deeper-living *S. altivelis* consistently incorporated more [^{32}P]ADP than the membranes from *S. alascanus*. Because the heterotrimeric holoprotein is the preferred substrate for the ribosylation reaction, the modulatory effects of the guanyl nucleotides GDP and GTPγS on ribosylation were assessed. GDP increased [^{32}P]ADP-ribosylation of the α subunits in *S. altivelis*. Only the highest concentration tested (1000 μM) increased [^{32}P]ADP-ribosylation in *S. alascanus* brain membranes to a slight extent. Increasing concentrations of GTPγS suppressed [^{32}P]ADP-ribosylation in *S. alascanus* brain membranes, presumably by promoting dissociation of the holotrimer. GTPγS had much less of an effect on the *S. altivelis* brain membranes. These differences in the extent of ADP-ribosylation and the modulatory effects of guanyl nucleotides may relate to the differential extent of coupling receptors to G

proteins in these two species. The effects of hydrostatic pressure on the PTX-catalyzed [^{32}P]ADP-ribosylation reaction differ in brain membranes of the two species. In the presence of GDP, the ribosylation reaction in *S. altivelis* is unaffected by pressures up to 360 atm. 68 atm of pressure inhibits ribosylation 40% in *S. alascanus* membranes.

SIGNIFICANCE: Increased hydrostatic pressure decreases basal adenylyl cyclase activity and decreases the efficacy of A_1 adenosine receptor agonists in modulating adenylyl cyclase in these species. The effects of increased hydrostatic pressure on transmembrane signal transduction by this system stem, at least in part, from pressure-increased GTP hydrolysis and the concomitant termination of inhibitory signal transduction, and the degree of coupling of the G protein pool to receptors. A general effect of pressure on agonist-receptor interactions with the G protein pool would be an important selective force in adaptation to the deep sea. Disruption of receptor coupling to effector elements via G proteins by pressure would be a critical impediment to colonization of the deep ocean by shallow-living organisms. Such a pressure-barrier to colonization would have important consequences both for the vertical distribution patterns of marine species, and the functioning of divers in this environment.

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